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Bone Quality Assessment in Individuals of Different Age, Gender and Body Constitution

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A B S T R A C T

The concept of bone quality describes the sets of the characteristics of the osseous tissue that influence bone strength. The aim was to explore the influence of anthropometric parameters and age on the parameters of the bone architecture and bone mineral properties in the lumbar vertebral bone of men and women. Vertebral bone samples underwent bone histomorphometry, bone densitometry and atomic absorption spectrometry. Men have greater values of the bone volume and thicker bone trabeculae in relation to women, which indicates that vertebral bone architecture is better preserved in men than in women. Age is the best predictor of changes that affect bone architecture and bone mineral properties. Bone mineral density value and calcium concentration are both negatively predicted by age, but positively predicted by boda mass index. Such result supports the opinion that low body mass index is associated with conditions of bone deficit such are osteopenia and osteoporosis.

Key words: *bone histomorphometry, anthropometry, bone densitometry, ash density*

Introduction

Bone strength correlates to the material and structural properties of bone tissue, as well as the direction of the biomechanical load that acts on it. The structural properties depend on the trabecular bone architecture, cortical bone thickness and porosity, while material properties of bone tissue are primarily dependent on the quality of collagen fibers and minerals incorporated in bone[1,2].

Initial changes that affect trabecular bone during aging are equal for both sexes and characterized by reduction of the value of bone volume and loss in trabecular bone thickness. After the age of sixty, age-dependent changes are characterized by really thinned bone trabeculae in men and reduced number of trabeculae and their weaker connectedness in women[3–8]. Loss of trabecular bone thickness does not significantly affect bone strength, while the loss of the trabecular number, significantly weakens the strength of trabecular bone[9,10]. The latter fact was confirmed in research that has proven that 10 % loss of bone mass as a consequence of the reduced number of bone trabeculae weakens the bone by 70%, compared to 20% weaker bone for the same bone mass deficit, which occurs by thinning of the bone trabeculae[9]. That the biomechanical ability of the lumbar vertebrae is gender dependent was previously established by Ebbesen and colleagues, who demonstrated that vertebrae of man can bear far greater mechanical load than vertebrae of women, which is explained with the fact that the bones of women are smaller compared to men[11].

Analysis of the iliac bone tissue biopsies has proven that women with vertebral fractures show changes in terms of low and high degree of bone tissue mineralization[12]. Hypomineralization of the bone tissue in osteomalacia[13] and osteoporosis[14] leads to physically weaker bones, which can not adequately respond to biomechanical loads.

The most widely used technique for diagnosis of osteoporosis is measurement of bone mineral density (BMD) by dual-energy X-ray absorptiometry (DXA). When it is not possible to apply bone densitometry, other parameters such as body mass index (BMI) becomes valuable in the assessment of osteoporosis. Low BMD is important predictor for spinal fracture as well as low BMI[15,16]. Even

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more, without information on BMD, the age-adjusted risk for any type of fracture increased significantly with lower BMI. Therefore, low BMI can be used to enhance the predictive value of BMD for the further fracture risk[15]. The relative influence of the bone volume fraction and ash fraction on the bone strength was previously investigated and results suggested that changes in mineral content generate a larger change in bone strength than a similar change in bone volume[17].

Results of the previously published investigations lack the information of the influence of anthropometry on the bone histomorphometric parameters. Thus, the objective of this study is to determine the influence of age and anthropometric parameters (body weight, body height and body mass index) on bone mass, structure and bone mineral properties in human lumbar vertebra.

Materials and Methods

The materials for this study comprised the whole body of the second (L2) and the third lumbar vertebra (L3). Half of the specimens were obtained from 44 women between 31 and 80 years of age at death and 44 from men between 30 and 79 years of age at death. The individuals had no history of musculoskeletal, malignant, liver or kidney disease.

The bodies of L2 and L3 were removed by cutting the vertebral arch, after which the specimen was frozen at –20 °C. The body of L3 was subsequently cleaned of soft tissue with a scalpel and vertical cylindrical sample was obtained by drilling with a bone trephine that has passed the entire height of the vertebral body. Each bone sample, consisting of the upper and lower end-plates and trabecular bone, was rinsed in saline, fixed in 4% paraformaldehyde, and embedded in methylmethacrylate without being decalcified. Two consecutive (5 μm thick) sections were cut every 150 μm using a Leica RM550 circular microtome (Leica, Vienna, Austria) equipped with a tungsten carbide knife. The sections were stained with toluidine blue and Masson trichrome, which gives a good contrast between bone and bone marrow. Data on height and weight of subjects were obtained from their health cards.

This study was approved by the local ethics committee.

Bone histomorphometry

The system for microscopy of the bone sections is consisted of Olympus BHA microscope (Olympus, Tokyo, Japan) and Pulnix digital camera (Pulmix, Yokohama, Japan) connected to the personal computer. Digital images were captured under 40X magnification and stored until the measurements were performed. For histomorphometric analysis a semiautomatic image analysis system, equipped with Issa software (VAMS, Zagreb, Croatia) was used.

According to the American Society of Bone and Mineral Research, trabecular bone volume (BV/TV, in %) corresponds to the amount of trabecular bone within the spongy space. It is derived from 2D measurements of bone area (B.Ar) and trabecular tissue area (T.Ar)[18] using Parfitt's formula, as follows: BV/TV = 100 B.Ar/ T.Ar.

Trabecular bone surface (BS/TV, in mm) was then calulated from the values of trabecular bone perimeter (B.Pm) and trabecular bone area (B.Ar)[18] : BS/TV= B.Pm/B.Ar.

In addition, three more values were calculated to evaluate the architecture of trabecular bone: (1) Trabecular thickness (Tb.Th, in μm) was derived from trabecular perimeter (B.Pm) and bone area (B.Ar) according to the Parfitt's formula[18]: Tb.Th=(B.Ar/B.Pm) (π/2); (2) Trabecular number (Tb.N, in mm) was derived from trabecular perimeter (B.Pm) and total tissue area (T.Ar) according to formula[18]: Tb.N=(B.Pm/T.Ar) * 10; (3) Trabecular separation (Tb.Sp, in μm) was derived according to the formula[18]: Tb.Sp=(1000 * T.Ar–B.Ar)/ B.Pm

Cortical parameters included cortical thickness (Ct. Th, in μm) and cortical bone volume (CV, in %). They were calculated according to the following formulas: Ct.Th =2 Pm * π/4 (where Pm is a cortical bone perimeter), and: CV= (C.Ar/TV) * 100 (where C.Ar is cortical bone area and TV is total tissue area).

Bone densitometry

Before being processed for histomorphometry, the body of L3 was immersed in a pot with a saline liquid and scanned using the device for bone densitometry (Hologic, Bedford, MA, USA).

By this analysis, necessary scans were obtained: anterior – posterior (AP) and lateral (L) and quantified by parameters: bone mineral content (BMC, mg) and bone mineral density (BMD, mg/cm^2).

Atomic absorption spectrometry

After being placed in a weighed porcelain pot, the body of L2 is weighed using electronic scales (Gilbertini Elettronica, Milano, Italy). In this way wet weight of the bone specimen was obtained. Bone specimens were dried by autoclave at 105 °C to constant weight, after which the electronic scale measured their dry weight. At the next step, bone specimens were burned in muffon oven to 800 °C for 48 hours with the temperature gradually lifting. The weight of dry matter obtained is expressed in g/mL. Part of the weighted dry matter was dissolved in 1 mL of concentrated nitric acid with heating on the hot plate, and then diluted with deionized water to 25 mL.

Samples for measurement are diluted to appropriate concentration and then in all samples and standards for measuring, 1% solution of Lantan (La^{3+}) is added in order to compensate for the influence of phosphate by measuring the AAS instrument (AA-375, Varian Instrument, Australia). Range of measurement standard of Ca concentration is from1 to 15 mg/L, while a range of measuring the concentration of Mg is from 0.1 to 0.5 mg/L. Concentrations of calcium (mg/L) and magnesium (mg/L) were measured at wavelength of 422.7 nm for calcium and at 285.2 nm for magnesium in the current flame of acetylene-air mixture.

Statistics

Statistica 7.1 (StatSoft) computer software (StatSoft Inc., Tulsa, OK) was used for statistical analyses. Data was tested for normal distribution and for each bone parameter student t test for independent groups was used to explore differences between men and women. Multiple regression analysis was employed to examine the influence of independent variables (age, body height, body weight and BMI) on dependent variables – parameters of: bone histomorphometry, bone densitometry and atomic absorption spectrometry. Results were considered statistically significant at the level $p<0.05$ and $p<0.01$.

Results

A descriptive analysis of the study population sample is presented in the Table 1. Men are significantly taller and heavier than women ($p<0.001$). With regard to given histomorphometric parameters, men had for 9.89% greater values of the trabecular bone volume and for 15.9% thicker bone trabeculae than women, with significance ($p<0.001$). All the values of parameters of the bone mineral properties (BMD, BMC, AD, Ca and Mg) were significantly higher in men than in women ($p<0.001$).

Only statistically significant independent variables are shown in Tables 2–5. These variables significantly affect all histomorphometric parameters, with the exception of the Ct.Th of the lower end-plate. The way independent variables affect the bone parameters was observed through the beta ponders. If β ponders are negative the relationship is inversely proportional, while the positive β means exactly proportional relationship.

Analysis of the impact of individual variables on bone parameters proved that age has a significant impact on the most parameters of lumbar vertebrae. Those are the parameters of the bone volume, bone structural parameters, cortical bone volume, cortical thickness of the upper end plate and bone mineral content ($p<0.01$). Bone mineral density is significantly affected by all variables, in a way that age and body weight have a negative impact, while body mass index and body height have a positive effect on BMD ($p<0.01$). The influence of independent variables on the calcium concentration is similar to the latter effect on BMD, so that age ($p<0.05$) and weight ($p<0.05$) have a negative impact, whereas BMI ($p<0.05$) has a positive effect on calcium (Table 3). Body height has a positive effect not only on the value of calcium ($p<0.01$), but also the density of vertebral bone dry matter ($p<0.05$).

Two separate analyses for men and women have proven significant impacts of independent variables (age, height, weight and BMI) on all bone parameters of the lumbar vertebrae, except for three parameters. These are the thickness of the lower end plate, the concentration of magnesium and ash density. Cortical bone volume, bone mineral content, and concentration of the calcium are significantly predicted in women, while the thickness of the upper end-plate is significantly predicted in men, by age and anthropometric parameters. Among other independent variables, only age significantly pre-

TABLE 1
DESCRIPTIVE CHARACTERISTICS OF THE STUDY PARTICIPANTS

Characteristics	Men (n=44)	Women (n=44)	p value
Age (years)	53±8.2	53.6±14.3	0.84
Body weight (kg)	81.27±10	68.3±7.8	<0.001
Body height (cm)	178.5±5.2	165.8±4.8	<0.001
Body mass index (kg/m^2)	25.4±2.3	24.9±3.1	0.38
BV/TV(%)	14.15±2.8	12.8±2.6	<0.05
BS/TV(/mm)	2.7±0.3	2.6±0.3	0.17
Tb.Th (μm)	112.3±12.5	95.4±11.1	<0.001
Tb.N (/mm)	1.3±0.2	1.3±0.1	0.86
Tb.Sp (μm)	652.3±103.6	669.9±99.1	0.42
CV lower endplate (%)	85.2±5.3	84.3±4.7	0.38
Ct.Th lower endplate (μm)	372.80±59.4	362.8±55.5	0.41
CV upper endplate (%)	84.8±5.2	83.5±5.3	0.27
Ct.Th upper endplate (μm)	398.7±53	382.6±61.3	0.19
BMD (g/cm^2)	0.46±0.1	0.36±0.1	<0.001
BMC (mg)	7.43±2	5.56±1.4	<0.001
AD (g/mL)	0.2±0.04	0.16±0.03	<0.001
Ca (mg/L)	0.07±0.02	0.06±0.02	<0.001
Mg (mg/L)	0.04±0.01	0.03±0.01	<0.001

TABLE 2
EFFECT OF AGE AND ANTHROPOMETRIC PARAMETERS ON PARAMETERS OF THE BONE ARCHITECTURE AND BONE MINERAL PROPERTIES

Dependent variables	Independent variables	R	R^2	F	P
BV/TV (%)	age body height body weight BMI	0.90	0.82	99.07	0.000
BS/TV (/mm)	age body height body weight BMI	0.74	0.55	25.76	0.000
Tb.Th (μm)	age body height body weight BMI	0.80	0.64	37.44	0.000
Tb.N (/mm)	age body height body weight BMI	0.75	0.57	27.7	0.000
Tb.Sp (μm)	age body height body weight BMI	0.78	0.62	34.16	0.000
CV(%) upper endplate	age body height body weight BMI	0.46	0.21	5.56	0.000
CV(%) lower endplate	age body height body weight BMI	0.63	0.39	13.47	0.000
Ct.Th (μm) gornja ploča	age body height body weight BMI	0.44	0.19	4,87	0.001
BMD (g/cm^2)	age body height body weight BMI	0.63	0.40	13.67	0.000
BMC (g)	age body height body weight BMI	0.57	0.32	9.97	0.000
AD (g/mL)	age body height body weight BMI	0.47	0.22	5.74	0.000
Ca (g/mL)	age body height body weight BMI	0.52	0.27	7.57	0.000
Mg (g/mL)	age body height body weight BMI	0.45	0.20	5,24	0.000

R – multiple correlation coefficient
R^2 – determination coefficient
p – statistical significance

dicted examined bone parameters. As a rule, age has a negative effect on given bone parameters, with the exception of Tb.Sp that affects positively (Table 5). Influence of age on bone parameters of the lumbar vertebrae differs in men and women. Thus, in women age significantly affects the concentration of calcium in the L2, whereas in men the impact of age on calcium was not significant (Table 5).

Discussion

Bone architecture and bone mineral properties

The architecture and the mineral properties of the vertebral bone are gender dependent. In terms of the bone histomorphometry men have greater values of the bone volume and thicker trabeculae than women. In terms of the bone mineral properties, then both: the values of the bone densitometry parameters (BMD, BMC) and the values of the bone absorption spectrometry parameters (AD, Ca, Mg) are greater in men than in women.

In the younger age the mean height and weight are greater in men than in women, which is why vertebral column in men carries the greater biomechanical load[19]. However, the amount of load that is distributed per unit area of vertebrae is equal in men and women, because a larger load schedules in greater bone area and vice versa. It was Mosekilde in her research that led to major findings of the bone volume in men should be taken with a grain of salt because this finding comes from their large bodies of the lumbar vertebrae[20]. Mean values of the body height and body weight measured in our men are larger than in women, suggesting that men have greater skeletal organs. Their higher values of bone volume, if you were to judge by what is presented by Mosekilde are natural phenomena of the higher and heavier sex. In another study Mosekilde has pointed to clear differences in trabecular bone structure between men and women, which are characterized by greater bone perforation of bone trabeculae in women compared to men[21]. Such changes which would indicate different mechanisms affecting trabecular bone structure were not found in this study. According to the theory presented by Parkinson et all, with the fall of the value of bone volume below 15%, resulting changes in the structure of skeletal bodies are irreparable. It is therefore possible that 9.89% greater values of the trabecular bone volume and 15.9% thicker bone trabeculae in men contribute to their better bone architecture, compared to women.

In studies that have been published, and are similar design as ours, the researchers have compared the values of bone mineral density and ash density of the lumbar vertebra with its ability to resist compressive loads. They got the result according to which the relationship bet-

TABLE 3
EFFECT OF THE SINGLE INDEPENDENT VARIABLES ON DEPENDENT VARIABLES

Dependent variables	Independent variables	β	r	p
BV/TV (%)	age	–0.809	–0.785	0.000
BS/TV (/mm)	age	–0.685	–0.705	0.000
Tb.Th (μm)	age	–0.504	–0.634	0.000
Tb.N (/mm)	age	–0.731	–0.735	0.000
Tb.Sp (μm)	age	0.731	0.755	0.000
CV (%) upper endplate	age	–0.436	–0.423	0.000
CV (%) lower endplate	age	–0.585	–0.589	0.000
Ct.Th (μm) upper endplate	age	–0.386	–0.375	0.000
BMD (g/cm^2)	age	–0.295	–0.346	0.001
	body height	1.787	0.282	0.008
	body weight	–2.166	–0.231	0.032
	BMI	1.813	0.268	0.012
BMC (g)	age	–0.279	–0.313	0.003
AD (g/mL)	body height	1.587	0.223	0.039
Ca (g/mL)	age	–0,229	–0.251	0.020
	body height	2,058	0.294	0.006
	body weight	–2.604	–0.251	0.020
	BMI	1.940	0.261	0.015

β – beta ponder
r – partial correlation coefficient
p – statistical significance

TABLE 4
EFFECT OF ANTHROPOMETRIC PARAMETERS AND AGE ON THE PARAMETERS OF THE BONE ARTICHETURE AND BONE MINERAL PROPERTIES IN MEN AND WOMEN

Dependent variables	Gender	Independent variables	R	R^2	F	p
BV/TV (%)	M FM	age body height body weight BMI	0.924 0.896	0.854 0.804	57.409 40.07	0.000 0.000
BS/TV (/mm)	M FM	age body height body weight BMI	0.780 0.717	0.608 0.515	15.156 10.365	0.000 0.000
Tb.Th (μm)	M FM	age body height body weight BMI	0.763 0.860	0.582 0.740	13.624 27.860	0.000 0.000
Tb.N (/mm)	M FM	age body height body weight BMI	0.761 0.815	0.579 0.664	13.453 19.334	0.000 0.000
Tb.Sp (μm)	M FM	age body height body weight BMI	0.770 0.857	0.593 0.735	14.260 27.119	0.000 0.000
CV(%) gornja ploča	FM	age body height body weight BMI	0.504	0.254	3.328	0.019
CV (%) lower endplate	M FM	age body height body weight BMI	0.624 0.656	0.390 0.430	6.236 7.376	0.000 0.000
Ct.Th (μm) upper enplate	M	age body height body weight BMI	0.533	0.284	3.884	0.009
BMD (g/cm²)	M FM	age body height body weight BMI	0.516 0.626	0.267 0.392	3.554 6.302	0.014 0.000
BMC (g)	FM	age body height body weight BMI	0.484	0.234	2.983	0.030
Ca (g/mL)	FM	age body height body weight BMI	0.464	0.215	2.684	0.045

R – multiple correlation coefficient
R^2 – determination coefficient
F – confidence limit
p – statistical significance
M – male
FM – female

TABLE 5
EFFECT OF THE SINGLE INDEPENDENT VARIABLES ON DEPENDENT VARIABLES

Dependent variables	Gender	Independent variables	β	r	p
BV/TV (%)	M	age	–0.884	–0.870	0.000
	F	age	–0.875	–0.902	0.000
BS/TV (/mm)	M	age	–0.735	–0.730	0,000
	F	age	–0.658	–0.641	0.000
Tb.Th (μm)	M	age	–0.591	–0.640	0.000
	F	dob	–0.879	–0.836	0.000
Tb.N (/mm)	M	age	–0.670	–0.685	0.000
	F	age	–0.783	–0.767	0.000
Tb.Sp (μm)	M	age	0.624	0.665	0.000
		body weight	–4.352	–0.318	0.042
		BMI	3.208	0.319	0.042
	F	age	0.845	0.823	0.000
CV (%)	M	age	–0,463	–0.428	0.005
upper endplate	F	age	–0.558	–0.496	0.000
CV (%)	M	age	–0.550	–0.540	0.000
lower endplate	F	age	–0.681	–0.623	0.000
Ct.Th (μm)	M	age	–0.560	–0.516	0.000
upper endplate	F	age	–0.342	–0.314	0.045
BMD (g/cm^2)	M	age	–0.421	–0.431	0.004
	F	age	–0.536	–0.495	0.001
BMC (g)	M	age	–0.409	–0.384	0.012
	F	age	–0.458	–0.420	0.006
Ca (g/mL)	F	age	–0.338	–0.319	0.041

β – beta ponder
r – partial correlation coefficient
p – statistical significance
M – male
F – female

ween bone strength and its mineral composition is stronger than the correlation between bone strength and bone volume[17]. According to the values of the parameters which describe bone mineral properties, our results indicate that men have stiffer vertebrae than women, which suggest that those skeletal organs are stronger in men, too.

Anthropometric parameters, age and lumbar vertebra

Anthropometric parameters and age significantly predict the course of the changes that affect architecture and mineral properties of the human lumbar vertebrae. Taking into account gender differences, the impact of age is lost on the value of calcium in men, but it is recorded that age is conversely proportional to the values of calcium in women.

The research results that are partially comparable with ours were obtained from clinical studies which examined the extent to which anthropometric parameters and age affect the value of calcaneus's bone ultrasound parameters, as well as the values of the parameters of bone densitometry[22–24]. Primary ultrasound parameters such as speed of sound (SOS) and ultrasound attenuation (BUA), provide information on the structural properties of bone, which is an advantage in assessing the bone quality, compared to the parameters of bone densitometry[24]. According to the results so far published, age has a negative, while BMI has a positive impact on the value of ultrasound bone parameters[22–24]. Besides the fact that the values of ultrasound parameters decrease with increasing porosity, as was proven in osteoporosis, our findings of the influence of age on parameters of bone architecture, in addition to being compliant with bone ultrasound measurements, speaks in favor of structural changes in the vertebrae. In fact, the opposite proportional relation of the age with the values of bone volume and trabecular bone thickness is a confirmation of our results that illustrate the changes that affect the architecture of the lumbar vertebra. Those are related to the architectural changes in lumbar vertebra, characterized by the fall of the value of bone volume and reduced trabecular bone thickness.

Women have smaller values of the parameters that describe the bone mineral properties in relation to men. When you look the latter fact in the context of reverse relations of the age to the values of calcium in women, it can be said that women are more exposed to processes that lead to a reduction in the value of minerals in the lumbar vertebra, compared to men.

Conclusion

Average values of bone mass were greater in men than in women. Analysis of the structure of bone tissue has proven that men have thicker bone trabeculae in relation to women. Histomorphometric parameters indicate that the architecture of bone tissue in the lumbar vertebra is better preserved in men than in women. Age is the best predictor of changes that affect architecture and mineral composition of the lumbar vertebra. Bone mineral density value and calcium concentration are both negatively predicted by age, but positively predicted by BMI. Such result supports the oppinion that low BMI is associated with conditions of bone deficit such are osteopenia and osteoporosis.

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PROCJENA KVALITETE KOSTI U OSOBA RAZLIČITE DOBI, SPOLA I TJELESNE KONSTITUCIJE

SAŽETAK

Ideja kvalitete kosti predstavlja skupne karakteristike koštanog tkiva koje utječu na snagu kosti. Cilj je bio istražiti utjecaj antropometrijskih parametara i starosne dobi na parametre koštane arhitekture te parametre mineralnih svojstava kosti u slabinskom kralješku žena i muškaraca. Uzorci kosti slabinskog kralješka analizirani su metodama: koštane histomorfometrije, koštane denzitometrije i atomske apsorpcijske spektrometrije. Muškarci imaju veće vrijednosti obujma kosti i debljine koštanih gredica u odnosu na žene, što upućuje na bolju održanost arhitekture kosti slabinskog kralješka u muškaraca. Starosna dob najbolji je pokazatelj promjena koje zahvaćaju arhitekturu i mineralna svojstva koštanog tkiva kralješka. Vrijednosti mineralne gustoće kosti, kao i koncentracija kacija obrnuto su razmjerne sa starosnom dobi, ali su upravo razmjerne s vrijednostima indeksa tjelesne mase. Potonji rezultat podržava teoriju da su niske vrijednosti indeksa tjelesne mase povezane sa stanjima koštanog deficita kao što su osteoporoza i osteopenija.